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(54) Title: PEPTIDE ANALOGUES OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) OR FACTOR 2 (IGF-2)

(57) Abstract

A peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2.

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PEPTIDE ANALOGUES OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) OR FACTOR 2 (IGF-2)

This invention relates to growth factors, related compounds and their use.

Insulin-like growth factor-1, a somatomedin, is a small protein that has been shown to stimulate growth of a wide range of cells in culture. Human IGF-1 (hIGF-1) has been purified to homogeneity from human serum and its complete amino acid sequence established. The serum mediator of growth hormone action, somatomedin C, has been shown to have an identical sequence to hIGF-1 so that these two are now considered as being synonymous. The amino acid sequence established for hIGF-1 beginning with the N-terminal glycine is:

Gly-pro-glu-thr-leu-cys-gly-ala-glu-leu-
val-asp-ala-leu-gin-phe-val-cys-gly-asp-
arg-gly-phe-tyr-phe-asn-lys-pro-thr-gly-
tyr-gly-ser-ser-ser-arg-arg-ala-pro-gin-
thr-gly-ile-val-asp-glu-cys-cys-phe-arg-
ser-cys-asp-leu-arg-arg-leu-glu-met-tyr-
cys-ala-pro-leu-lys-pro-ala-lys-ser-ala-

Bovine IGF-1 and porcine IGF-1 have identical sequences.

Using the conventional numbering system of the N-terminal glycine being residue #1 and the C-terminal alanine residue #70, ovine and chicken IGF-1 differ from human IGF-1 only as follows:

ovine IGF-1 : ala^{66}

Chicken IGF-1 : ser^{26}; lys^{41}, gln^{50}, ile^{64}

IGF-1 levels in serum correlate positively with growth rates in boys during adolescence and negatively with the

degree of growth hormone deficiency in growth-retarded subjects, and to both growth rate and eventual size in mice transfected with growth hormone genes. These findings, indirectly linking IGF-1 concentrations with growth rates and supported by more direct evidence that administration of IGF-1 leads to restoration of growth rates in hypopituitary (growth hormone deficient) rats or mice and to increased growth rates in normal rats, have lead to the interpretation that IGF-1 might usefully be applied: (1) in humans to treat growth hormone deficiencies; (2) in farm animals to increase growth rates, increase the relative proportion of muscle and enhance food conversion efficiency. It is further suggested that administration of IGF-1: (3) may suppress the loss of body protein in severe human catabolic states such as following burns, infection or other trauma; (4) may improve wound healing in human subjects as well as in animals. IGF-1 can also be used to (5) support the growth of cells in culture.

The result of the above inferences is that there is a commercial demand for IGF-1 for use in animal trials, clinical investigations and for cell culture. However, only milligram amounts of hIGF-1, for example, are available by purification of tonnes of human serum protein and yields from recombinant DNA methods remain low.

Insulin-like growth factor-2 (IGF-2) like IGF-1, is a small protein that has been shown to stimulate growth of cells in culture. In most cases, these biological effects occur following interaction of IGF-2 with the same cellular receptor as is involved in IGF-1 actions. The amino acid

sequence established for human IGF-2 (hIGF-2) beginning with the N-terminal alanine is shown below. Upper case letters have been used to indicate the amino acids equivalent to the N-terminal 5 amino acids of hIGF-1:

Ala-tyr-arg-PRO-SER-GLU-THR-LEU-cys-gly-
gly-glu-leu-val-asp-thr-leu-gln-phe-val-
cys-gly-asp-arg-gly-phe-tyr-phe-ser-arg-
pro-ala-ser-arg-val-ser-arg-arg-ser-arg-
gly-ile-val-glu-glu-cys-cys-phe-arg-ser-
cys-asp-leu-ala-leu-leu-glu-thr-tyr-cys-
ala-thr-pro-ala-lys-ser-glu

Using the conventional numbering system of the N-terminal alanine being residue #1 and the C-terminal glutamic acid being residue #67, bovine, ovine, porcine and chicken IGF-2 differ from human IGF-2 only as follows:

bovine IGF-2 : ser^{32}; ile^{35}; asn^{36}
ovine IGF-2 : ser^{32}; ile^{35}; asn^{36}; ala^{62}
porcine IGF-2 : asn^{36}
chicken IGF-2 : ala^{1} missing; gly^{3}; thr^{4}; ala^{5}; val^{32}; gly^{33}; asn^{35}; asn^{36}; ile^{39}; asn^{40}

It has been disclosed (see PCT/AU87/00246 to applicants) that compounds corresponding to IGF-1 but lacking one to five, preferably three amino acid residues from the N-terminal of the molecule can exhibit a substantial increase in biological potency compared with the more complete compounds.

For example, the compound destripeptide bIGF-1 but lacking the amino acid residues gly, pro and glu from the

N-terminal, is effective in inhibiting protein breakdown and stimulating both protein synthesis and DNA synthesis in cellular systems at concentrations between 4 and 50 fold lower than required for entire bIGF-1.

For IGF-1 peptides having N-terminal amino acid sequences in common with that of human/bovine/porcine IGF-1, the elimination of between 1 and 5 amino acid residues from the N-terminal also results in enchanced biological potencies. The said N-terminal amino acid sequence is also a feature of the IGF-1 of rat, ovine, and chicken species.

However, a useful property of the full IGF-1 peptide but not shared by the IGF-1 peptides having 1 to 5 N-terminal amino acids eliminated is that production by recombinant DNA methods that are part of the prior art are facilitated by the existence of N-terminal glycine. This facilitation occurs because an asparagine residue can be engineered upstream from the glycine and the asparagine/glycine bond cleaved selectively by mild hydroxylamine treatment following expression of the engineered gene.

Accordingly it is an object of the present invention to overcome, or at least alleviate, one or more of the difficulties related to the prior art.

Accordingly in a first aspect of the present invention there is provided a peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2. It will be understood that in respect

of chicken IGF-2 the N-terminal Ala-residue is absent so that the glutamic acid residue is at position 5 from the N-terminal.

Preferably the peptide analogue is a human, bovine, ovine, porcine or chicken insulin-like growth factor analogue. More preferably the peptide analogue is a human, bovine or porcine insulin-like growth factor-1 analogue.

The peptide analogue according to the present invention may be in a biologically pure form.

In a preferred aspect of the present invention, wherein the peptide analogue, is an insulin like growth factor-1 analogue in addition at least one of the Gly-, Pro-, or Thr- residues may be absent from the N-terminal in addition to the absence of the glutamic acid residue.

In a preferred aspect of the present invention the glutamic acid residue may be replaced by a different amino acid residue.

Suitable amino acid residues to replace glutamic acid include glycine, glutamine, leucine, arginine, or lysine.

More preferably the replacement residue for glutamic acid will be a positively charged amino acid residue such as arginine or lysine. Alternatively the glutamic acid residue may be replaced by glycine and the threonine residue normally adjacent to the glutamic acid may be replaced by a different amino acid residue, preferably arginine or glycine, most preferably arginine.

Preferably the N-terminal sequence is selected from

Val-Leu-Cys-

Arg-Leu-Cys-

Gly-Leu-Cys-

Gly-Thr-Leu-Cys-

Gly-Pro-Arg-Thr-Leu-Cys-

Gly-Pro-Gly-Arg-Leu-Cys-

Gly-Pro-Gly-Gly-Leu-Cys-

Gly-Pro-Gly-Thr-Leu-Cys-

Gly-Pro-Gln-Thr-Leu-Cys-

Gly-Pro-Lys-Thr-Leu-Cys-

Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 6 from the N-terminal.

In a further preferred aspect of the present invention the peptide analogue is an insulin-like growth factor-2 analogue. Preferably in the peptide analogue, at least one of the Ala-, Tyr-, Arg-, Pro-, Ser- or Thr- residues is absent from the N-terminal in addition to the absence of the glutamic acid residue.

More preferably the glutamic acid residue is replaced by a different amino acid residue.

Suitable amino acid residues to replace glutamic acid include glycine, glutamine, leucine, arginine, or lysine. Suitable amino acid residues to replace the threonine residue include arginine or glycine.

More preferably the replacement residue for glutamic acid will be a positively charged amino acid residue such as arginine or lysine. Alternatively the glutamic acid residue may be replaced by glycine and the threonine residue

normally adjacent to the glutamic acid may be replaced by a different amino acid residue, preferably arginine or glycine, most preferably arginine.

Preferably the N-terminal sequence is selected from

Ala-Tyr-Arg-Pro-Ser-Lys-Thr-Leu-Cys-

Ala-Tyr-Arg-Pro-Ser-Arg-Thr-Leu-Cys-

Ala-Tyr-Arg-Pro-Ser-Gly-Arg-Leu-Cys-

Ala-Tyr-Arg-Pro-Ser-Gly-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 9 from the N-terminal.

The peptides lacking the glutamic acid residue bind poorly to the binding proteins produced by many cell types. This binding may be further reduced by the substitution of an arginine or lysine residue for the glutamic acid residue and optionally the substitution of the adjacent threonine residue by arginine or a lysine residue. Should a binding protein be present those other IGF-1 peptides that do bind have reduced potencies.

In a preferred aspect wherein the glutamic acid residue is either substituted by another amino acid or eliminated and the N-terminal residue is glycine, this invention provides peptide analogues that are suitable for cleavage of an engineered upstream asparagine. The peptide analogues have higher potencies than IGF-1 in cultured cells.

The peptide analogues according to the present invention may form suitable replacements for IGF-1 and -2 in the following applications: (1) in humans to treat growth hormone deficiencies; (2) in farm animals to increase growth rates, increase the relative proportion of muscle or

improve food conversion efficiency; (3) in humans to suppress the loss of body protein in severe catabolic states such as following burns, infection or other trauma; (4) in humans and animals to improve wound healing, and (5) to support the growth of cells in culture:

More specifically, the present invention provides a pharmaceutical or veterinary composition that includes:

(a) an effective amount of a peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 6 from the N-terminal of IGF-2 respectively and

(b) a pharmaceutically or veterinarily acceptable diluent, carrier or excipient therefor.

The peptide analogue may be present in amounts sufficient to provide a dose rate of approximately 0.01 to 10, preferably 0.1 to 1 milligrams/kg body weight/day. The peptide analogue may be present in amounts of from approximately 0.02 to 2000 milligrams. For cell culture applications the peptide analogue may be present in concentrations from approximately 0.1 to 100 milligrams per litre.

In a further preferred aspect of the present invention there is provided a method for the treatment of protein accumulation deficiencies or protein loss in human subjects, which method includes

administering to a patient to be treated an effective amount of a peptide analogue of insulin-like growth factor-1

(IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 6 from the N-terminal of IGF-2 respectively.

The peptide analogues may be administered to human subjects as a treatment for disorders associated with tissue wasting including, but not limited to, burns, skeletal trauma, infection, cancer, cystic fibrosis, Duchenne muscular dystrophy, Becker dystrophy, autosomal recessive dystrophy, polymyositis as well as other myopathies and acquired immune deficiency syndrome (AIDS). The peptide analogues may be administered parenterally or by injection.

In an alternative aspect there is provided a method for the treatment of wounds in animals including humans, which method includes

administering to a patient to be treated an effective amount of a peptide analogue of mammalian insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 6 from the N-terminal of IGF-2 respectively.

For the treatment of wounds in human subjects or in animals the peptide analogue may be applied externally to the wound or it may be administered by injection.

In a still further aspect there is provided a method for the improvement of growth performance in animals which method includes

administering to an animal to be treated an effective amount of a peptide analogue of insulin-like growth factor-1

(IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 6 from the N-terminal of IGF-2 respectively.

An implant, preferably a slow release pellet, is the preferred method of administration to farm animals as applied in conventional practice. Otherwise the peptide analogue may be administered by injection.

The peptide analogues of the present invention may be administered to premature or other human infants to promote growth, improve nitrogen status and to treat catabolic disorders. The peptides may be administered as outlined above for tissue wasting conditions.

Accordingly in a still further aspect of the present invention, there is provided a method for the stimulation of cells in culture which method includes

providing

a culture medium, and

an effective amount of a peptide analogue as described above; and

adding the peptide analogue to the culture medium.

Any standard culture medium may be used in accordance with this aspect of the present invention. For example the culture medium may include Eagle's Minimal Essential Medium.

In a further aspect of the present invention there is provided a method for the preparation of a peptide analogue of IGF-1 wherein at least the glutamic acid residue is absent at position 3 from the N-terminal which method includes

providing a source of amino acids, and

coupling the amino acids in sequence to form a peptide analogue having an N-terminal sequence selected from,

Val-Leu-Cys-

Arg-Leu-Cys-

Gly-Leu-Cys-

Gly-Thr-Leu-Cys-

Gly-Pro-Arg-Thr-Leu-Cys-

Gly-Pro-Gly-Arg-Leu-Cys-

Gly-Pro-Gly-Gly-Leu-Cys-

Gly-Pro-Gly-Thr-Leu-Cys-

Gly-Pro-Gln-Thr-Leu-Cys-

Gly-Pro-Lys-Thr-Leu-Cys-

Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue being that normally at position 6 from the N-terminal.

The peptide analogues may be produced by appropriate modifications to methods existing for the production of the full IGF-1 peptide. these modifications would be familiar to those familiar with the art.

Specifically, the peptides related to human/bovine/ porcine IGF-1 may be synthesisted chemically using procedures developed for human IGF-1 (for example: Li et al., Proc. Natl. Acad. Sci, USA 80: 2216-2220, 1983) but with the final cycles of amino acid ligation modified. Synthetic ovine or chicken IGF-1 as well as related IGF-1 and IGF-2 peptides may be produced by techniques similar to those used for human IGF-1 using amino acid sequence information for these peptides.

In accordance with the present invention, the peptides may also be produced following transformation of susceptible bacterial, yeast or tissue culture cell hosts with recombinant plasmids that include DNA sequences capable of directing the expression of the peptides. The DNA sequence may be synthetic, chromosomal, cDNA or combination thereof. The inserted coding sequences may incorporate deletion or omissions to account for differences between the sequence of peptide analogues and the full IGF-1 peptide.

The present invention will now be more fully described with respect to production of certain IGF-1 peptides and information on their biological potencies. It should be understood however, that the following description is illustrative only and should not be taken in any way as a restriction on the generality of the description foregoing.

Example 1

Synthesis of IGF-1 peptides

Chemical synthesis of human/bovine/porcine IGF-1 peptides with between 1 and 4 amino acids from the normal N-terminal modified has been effected by the following procedure.

The starting material was Boc-ala-phenylacetamido methyl resin. Coupling was effected in an Applied Biosystems Inc model 430A peptide synthesiser with preformed symmetric anhydrides of the Boc-aminoacids in dichloro-methane except for the derivatives of arginine, asparagine and glutamine which were coupled in dimethyl formamide (DMF). In all cases a second coupling was performed in DMF. Samples of resin were removed after each cycle of

synthesis and subjected to quantitive ninhydrin analysis (Sarin, V.K., Kent, S.B.H., Tam, J.P., Merifield, R.B.; Anal. Biochem. 17, 147-157 (1981). Preview sequence analysis of the side-chain protected, resin-bound peptide was also carried out and together, these indicated an average repetitive yield of 99%.

Portions of resin containing side-chain protected peptides corresponding to the complete sequence of hIGF-1 but with 4 to 0 amino acids not coupled at the N-terminal were removed. Other portions with between 4 and 3 amino acids not coupled at the N-terminal had amino acid residues coupled as required for specific analogues. Peptides were cleaved and deprotected according to Applied Biosystems Inc procedures and recovered as ether precipitates.

Peptides were redissolved in 6M guanidine hydrochloride pH 8.5 with Tris containing 10 mM dithioerythritol and desalted by reverse phase HPLC and dried. Oxidation of the reduced peptide was effected by dissolving in 8M urea, 0.1M Tris (pH8.0 with HCl) containing 13mM oxidized gluthathione and incubated at 25^{o} for 15 hours. The sample was purified by reverse phase HPLC using a gradient of acetonitrile in 0.1% trifluoroacetic acid to elute the peptides and separate the biologically active form of the peptide from those forms lacking the correct disulphide bonds and hence lacking full biological activity. The samples were dried prior to resuspension.

Biological activity was confirmed by the ability of the peptide to stimulate protein synthesis in L6 myoblasts.

It will be appreciated that various modifications

and/or alterations may be introduced into the constructions and arrangementsof parts previouslydescribed without departing from the spirit or ambit of the present invention.

Biological Activities of IGF-1 Peptides

Bioassays of purified synthetic peptides have been compared with pure humna/bovine/porcine IGF-1. The assays involve the incorporation of ^{3}H-labelled leucine into the total cell protein of L6 myoblasts as described by Francis et al. (Biochem. J. 233:207-213, 1986). The relative potencies are shown in Table 1 where the concentrations are expressed as percentages of that required to give a half-response with human/bovine/porcine IGF-1 (12 ng/ml):

TABLE 1

Relative biological potencies of IGF-1 peptides in L6 myoblasts

N-terminal sequence (the Cys residue is that normally at position 6 from the N-terminal)	Concentration (percent of that required to give a half-response with hIGF-1)
Human/bovine/porcine IGF-1 (Gly-Pro-Glu-Thr-Leu-Cys-)	100
Thr-Leu-Cys-	15
Val-Leu-Cys-	13
Gly-Leu-Cys-	15
Arg-Leu-Cys-	4
Gly-Thr-Leu-Cys-	16
Gly-Pro-Gly-Thr-Leu-Cys-	12
Gly-Pro-Gln-Thr-Leu-Cys-	18
Gly-Pro-Lys-Thr-Leu-Cys-	11
Gly-Pro-Leu-Thr-Leu-Cys-	18
Gly-Pro-Arg-Thr-Leu-Cys-	5
Gly-Pro-Gly-Gly-Leu-Cys	14
Gly-Pro-Gly-Arg-Leu-Cys	4

The higher potencies produced by the deletion or modification of the glutamic acid residue normally at position 3 of IGF-1 are not associated with a markedly

increased competition of the peptide for binding to receptors on the L6 myoblasts, provided that binding is carried out at 4°C otherwise using the method of Ballard et al (Biochem. J. 233; 223-230, 1986) with human/bovine/porcine IGF-1 as radioligand. This apparent discrepancy is caused by the myoblasts producing a binding protein in addition to the receptor. This binding protein selectively binds those IGF-1 peptides that have a glutamic acid residue at position 3 from the N-terminal, thus preventing the peptide binding to the cell receptor. This interpretation is established by the following results [determined by the method of Martin and Baxter, J. Biol, Chem. 261: 8754-8760, (1986)] with purified binding proteins (see Table 2).

TABLE 2

Relative abilities of IGF-1 Peptides to complete for the binding of labelled human/bovine/porcine IGF-1 to purified binding protein

N-terminal sequence (the Cys residue is that normally at position 6 from the N-terminal)	Potency for binding to the protein where hIGF-1 = 100%
Human/bovine IGF-1	
(Gly-Pro-Glu-Thr-Leu-Cys-)	100
Thr-Leu-Cys-	0.2
Val-Leu-Cys-	0.1
Gly-Leu-Cys-	0.1
Arg-Leu-Cys	< 0.1
Gly-Thr-Leu-Cys-	1.0
Gly-Pro-Gly-Thr-Leu-Cys-	0.5
Gly-Pro-Gln-Thr-Leu-Cys-	1.0
Gly-Pro-Lys-Thr-Leu-Cys-	0.1
Gly-Pro-Leu-Thr-Leu-Cys-	0.5
Gly-Pro-Arg-Thr-Leu-Cys-	< 0.1
Gly-Pro-Gly-Gly-Leu-Cys-	0.1
Gly-Pro-Gly-Arg-Leu-Cys-	< 0.1

Accordingly, from the data presented in Tables 1 and 2 it can be seen that:

- removal of the three N-terminal amino acids (Gly,Pro,Glu) from hIGF-1 leads to enhanced biological activity and minimal binding to binding proteins produced by the cells;
- removal of the three N-terminal amino acids together with substitution of the fourth amino acid (threonine) with arginine gives even greater biological potency as well as even lower binding to the binding proteins;
- removal of the two N-terminal amino acids (Gly,Pro) together with the substitution of the third amino acid (glutamic acid) with glycine leads to an IGF analogue that is more active biologically than hIGF-1 and binds poorly but significantly to the binding proteins;
- substitution of the Glutamic acid residue normally at position 3 in hIGF-1 with glycine, glutamine, lysine, leucine or arginine leads to enhanced potency and poor binding to binding proteins, with the effects greatest with the lysine or arginine substitutions;
- substitution of the glutamic acid residue normally at position 3 in hIGF-1 with glycine together with substitutions of glycine, arginine or valine for threonine at position 4 also produce increases in potency and decreased binding to binding proteins.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

1. A peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2.

2. A peptide analogue according to claim 1 which is a human, bovine, ovine, porcine or chicken insulin-like growth factor analogue.

3. A peptide analogue according to claim 2 which is a human, bovine or porcine insulin-like growth factor-1 analogue.

4. A peptide analogue according to claim 3 wherein in addition at least one of the Gly-, Pro-, or Thr- residues is absent from the N-terminal.

5. A peptide analogue according to claim 3 wherein the glutamic acid residue is replaced by a different amino acid residue.

6. A peptide analogue according to claim 5 wherein the amino acid residue is replaced by a positively charged amino acid residue.

7. A peptide analogue according to claim 6 wherein the threonine residue normally adjacent the glutamic acid residue is replaced by a different amino acid residue.

8. A peptide analogue according to claim 1 having an N-terminal sequence selected from

Val-Leu-Cys-

Arg-Leu-Cys-

Gly-Leu-Cys-

Gly-Thr-Leu-Cys-

Gly-Pro-Arg-Thr-Leu-Cys-

Gly-Pro-Gly-Arg-Leu-Cys-

Gly-Pro-Gly-Gly-Leu-Cys-

Gly-Pro-Gly-Thr-Leu-Cys-

Gly-Pro-Gln-Thr-Leu-Cys-

Gly-Pro-Lys-Thr-Leu-Cys-

Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 6 from the N-terminal.

9. A peptide analogue according to claim 1 which is suitable for cleavage of an engineered upstream asparagine residue, including a terminal glycine residue.

10. A peptide analogue according to claim 1 in a biologically pure form.

11. A peptide analogue according to claim 1 which is a human, bovine, ovine, porcine or chicken insulin-like growth factor-2 analogue (IGF-2).

12. A peptide analogue according to claim 11 wherein in addition at least one of the Ala-, Tyr-, Arg-, Pro-, Ser- or Thr- residues is absent from the N-terminal.

13. A peptide analogue according to claim 11 wherein the glutamic residue is replaced by a different amino acid residue.

14. A peptide analogue according to claim 13 wherein the amino acid residue is replaced by a positively charged amino acid residue.

15. A peptide analogue according to claim 14 wherein the threonine residue normally adjacent the glutamic acid residue is replaced by a different amino acid residue.

16. A peptide analogue according to claim 13 having an

N-terminal sequence selected from

Ala-Thr-Arg-Pro-Ser-Lys-Thr-Leu-Cys-

Ala-Tyr-Arg-Pro-Ser-Arg-Thr-Leu-Cys-

Ala-Tyr-Arg-Pro-Ser-Gly-Arg-Leu-Cys-

Ala-Tyr-Arg-Pro-Ser-Gly-Thr-Leu-Cys-

with the Cys residue shown being that normally at position 9 from the N-terminal.

17. A peptide analogue according to claim 11 suitable for cleavage of an engineered upstream asparagine residue, including a terminal glycine residue.

18. A pharmaceutical or veterinary composition for the treatment of protein accumulation deficiencies or protein loss in animals including

(a) an effective amount of a peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2 and

(b) a pharmaceutically or veterinarily acceptable diluent carrier or excipient therefor.

19. A pharmaceutical or veterinary composition according to claim 18 wherein the glutamic acid residue in the peptide analogue is substituted by a different amino acid residue.

20. A pharmaceutical or veterinary compositoin according to claim 19 wherein the peptide analogue is present in amounts of from approximately 0.02 to 2000 milligrams.

21. A method for the treatment of protein accumulation deficiencies or protein loss in human subjects, which method includes

administering to a patient to be treated an effective amount of a peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2.

22. A method according to claim 21 wherein the glutamic acid residue in the peptide analogue is substituted by a different amino acid.

23. A method according to claim 22 wherein the protein accumulation defiencies are associated with infant prematurity, growth hormone deficiency, burns, infection, other trauma, cancer, cystic fibrosis, Duchenne muscular dystrophy, Becker dystrophy, autosomal recessive dystrophy, polymyositis, as well as other myopathies.

24. A method according to claim 23 wherein the peptide analogue is administered at a dose rate of approximately 0.01 to 10 milligrams per kilogram body weight per day.

25. A method for the treatment of wounds in animals including humans, which method includes

administering to a patient to be treated an effective amount of a peptide analogue of mammalian insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2.

26. A method according to claim 25 wherein the glutamic acid residue in the peptide analogue is substituted by a different amino acid residue.

27. A method according to claim 26 wherein the peptide

analogue is administered by injection or is applied externally in amounts of approximately 0.02 to 2000 milligrams.

28. A method for the improvement of growth performance in animals which method includes

administering to an animal to be treated an effective amount of a peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2.

29. A method according to claim 28 wherein improved growth performance is associated with increased growth rate, selective deposition of muscle, reduced amount of fat or increased food conversion efficiency.

30. A method according to claim 29 wherein the peptide analogue is administered at a dose rate of approximately 0.01 to 10 milligrams/kg body weight/day.

31. A method for improviing the growth of cells in culture which method includes

providing a culture medium and an effective amount of a peptide analogue of insulin-like growth factor-1 (IGF-1) or factor-2 (IGF-2) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal of IGF-1 or at position 5 or 6 from the N-terminal of IGF-2; and

adding the peptide analogue to the culture medium.

32. A method according to claim 31 wherein the peptide analogue is added in an amount of approximately 0.1 to 100 milligrams per litre of culture medium.

33. A method for the preparation of a peptide analogue of

insulin-like growth factor-1 (IGF-1) wherein at least the glutamic acid residue is absent at position 3 from the N-terminal which method includes

providing a source of amino acids, and

coupling the amino acids in sequence to form a peptide analogue having an N-terminal sequence selected from,

Val-Leu-Cys-

Arg-Leu-Cys-

Gly-Leu-Cys-

Gly-Thr-Leu-Cys-

Gly-Pro-Arg-Thr-Leu-Cys-

Gly-Pro-Gly-Arg-Leu-Cys-

Gly-Pro-Gly-Gly-Leu-Cys-

Gly-Pro-Gly-Thr-Leu-Cys-

Gly-Pro-Gln-Thr-Leu-Cys-

Gly-Pro-Lys-Thr-Leu-Cys-

Gly-Pro-Leu-Thr-Leu-Cys-

with the Cys residue being that normally at position 6 from the N-terminal.

34. A method according to claim 33 substantially as hereinbefore particularly described with reference to example 1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 88/00485

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C07K 7/10, C07K 7/40, A61K 37/36

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
	DERWENT WPI/WPIL; USPA Keywords : "INSULIN-LIKE GROWTH FACTOR" or "SOMATOMEDIN"

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU : C07C 103/52, C07K 7/10, 7/40

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y	AU,A, 62869/86 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION; BALLARD, Francis John et al.) 10 March 1987 (10.03.87) (see whole document).	(1-4,9,10,18, 21,25,28-32)
X	AU,A, 66330/86 (KABIGEN AB; VICKI RUBIAN SARA) 18 June 1987 (18.06.87) (see whole document).	(1-4,9,10,18, 21,25,28-32)
X	CHEMICAL ABSTRACTS, Vol.105, no.5, issued 1986, August 4, (Columbus, Ohio U.S.A.), Carlsson-Skwirut, Cristine, et al., 'Isolation and Characterisation of Variant IGF-1 as well as IGF-2 from Adult Human Brain', page 147, abstract no. 36363w, FEBS Lett. 1986, 201(1), 46-50.	(1-4,9,10,18, 21,25,28-32)
X	CHEMICAL ABSTRACTS, Vol.105, no.9, issued 1986, September 1, (Columbus, Ohio, U.S.A.), Sara, Vicki R., et al., 'Characterisation of Somatomedins from Human Fetal Brain: Identification of a Variant Form of Insulin-like Growth Factor I', page 142, abstract no. 73328j, PROC. NATL. ACAD. SCI. U.S.A. 1986, 83(13), 4904-7 (Eng).	(1-4,9,10,18, 21,25,28-32)

(continued)

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
7 April 1989 (07.04.89)	06 APRIL 1989 (06.04.89)
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	R. GRANT

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	CHEMICAL ABSTRACTS, Vol.106, no.13, issued 1987, March 30, (Columbus, Ohio, U.S.A.), Carlsson-Skwirut, C., et al., 'Circulating forms of human fetal somatomedin.', page 156, abstract no. 96902b, ACTA ENDOCRINOL. (Copenhagen) 1987, 114(1), 37-40 (Eng).	(1-4,9,10,18, 21,25,28-32)
Y	CHEMICAL ABSTRACTS, Vol.104, no.23, issued 1986, June 9, (Columbus, Ohio, U.S.A.), Blumberg, Shmaryahu, et al., 'Removing N-terminal amino acid residues from eukaryotic polypeptide analogues and polypeptides produced by this method.', page 410, abstract no. 203501c, PCT Int. Appl. WO 86 01,229, 27 Feb 1986, US Appl. 641,488, 16 Aug 1984.	(1-4,8-12)
P,X	CHEMICAL ABSTRACTS, Vol.108, no.23, issued 1988, June 6, (Columbus, Ohio, U.S.A.), Francis, Geoffrey L., et al., 'Insulin-like growth factors 1 and 2 in bovine colostrum. Sequences and biological activities compared with those of a potent truncated form.', page 82, abstract no. 198502e, BIOCHEM.J., 1988, 251(1), 95-103 (Eng).	(1-4,9,10,18, 21,25,28-32)
P,X	CHEMICAL ABSTRACTS, Vol.108, no.23, issued 1988, June 6, (Columbus, Ohio, U.S.A.) Dawe, S.R. et al., 'Purification, partial sequences and properties of chicken insulin-like growth factors.', page 421, abstract no. 202006r, J.ENDOCRINOL. 1988 117(2), 173-81 (Eng).	(1,2,10-14, 16)
P,X	CHEMICAL ABSTRACTS, Vol.109, no.3, issued 1988, July 18, (Columbus, Ohio, U.S.A.), Bayne, Marvin L., et al., 'Structural analogs of human insulin-like growth factor I with reduced affinity for serum binding proteins and the type 2 insulin-like growth factor receptor.', page 68, abstract no. 17150r, J.BIOL.CHEM. 1988, 263(13), 6233-9 (Eng).	(1,2,5-7, 19,20)
P,X	CHEMICAL ABSTRACTS, Vol.109, no.11, issued 1988, September 12, (Columbus, Ohio, U.S.A.), Cascieri, Margaret A., et al., 'Serum half-life and biological activity of mutants of insulin-like growth factor I which do not bind to serum binding proteins.', page 146, abstract no. 86924h, ENDOCRINOLOGY, (Baltimore) 1988, 123(1), 373-81 (Eng).	(1,2,5-7, 19,22)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 88/00485

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 62869/86	WO 8701038	EP 235205	
AU 66330/86	DK 6005/86 SE 8505920	EP 227619	JP 62187500

END OF ANNEX